

IN THE CLAIMS:

1. (currently amended) ~~An enzymatic process to obtain~~ A method for producing 4-O- β -D-galactopyranosyl-D-xylose enzymatically that comprises:

~~a first step of preparation of~~ (i) preparing a first reaction mixture of 2-20% by weight of D-xylose 0-5-5% by weight of a β -D-galactopyranoside substrate 75-97.5% by weight of a reaction medium that comprises buffered water at a pH between 5.0 and 9.0; adding 10 to 1,000 units of a β -D-galactosidase enzyme, per gram of β -D-galactopyranoside, to the first reaction mixture; and obtaining a second reaction mixture; ~~a second step wherein the second reaction mixture is~~ subjected

(ii) subjecting the second reaction mixture to a reaction at a temperature comprised between a temperature higher than the freezing point of the second reaction mixture and 45° C., for 2 to 48 hours, in order to form disaccharides in the second reaction mixture;

(iii) ~~a third step wherein the reaction is stopped~~ stopping the reaction when the disaccharides have been formed in the desired amount, by means of a treatment ~~chosen between~~ selected from the group consisting of deactivation of β -D-galactosidase by freezing the second reaction mixture at a temperature between -20 ° C. and -170 ° C., deactivation of β -D-galactosidase by heating the second reaction mixture at a temperature between 95 and 110 ° C., and separation of β -D-galactosidase from the second reaction mixture by ultrafiltration; obtaining a third reaction mixture;

(iv) ~~a fourth step wherein~~ separating an aglyconic fragment of the β -D-galactopyranoside substrate used in the first step ~~is separated~~ from the third reaction mixture by extraction or filtration; obtaining a fourth reaction mixture;

~~(v) a fifth step comprising isolation of~~ isolating fractions that contain 4-O- β -D-galactopyranosyl-D-xylose, ~~characterized in that, the fifth step is selected between~~ by a method selected from the group consisting of addition of celite to the fourth reaction mixture, followed by solid-liquid extraction with a solvent and elution with a first eluent in a column; and directly adding active carbon to the fourth reaction mixture followed by filtration and elution with a second eluent, ~~and in that, in a sixth step,~~

~~(vi) crystallizing the fractions that contain 4-O- β -D-galactopyranosyl-D-xylose, are crystallized~~ in a crystallization mixture selected ~~among~~ from the group consisting of mixtures of acetone/methanol in a ratio between 5/1 to 20/1 and mixtures of acetone/water in a ratio between 5/1 to 20/1.

2. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the fourth reaction mixture is concentrated before being subjected to elution in the column.

3. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the mixture of acetone/methanol has a ratio of 10/1.

4. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the mixture of acetone/water has a ratio of 10/1.

5. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the first eluent is a mixture of water/isopropanol that contains 1 to 10% (v/v) of isopropanol.

6. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the mixture of water/isopropanol contains 2% (v/v) of isopropanol.

7. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ ~~the fifth step (v)~~ consists of adding celite to the fourth reaction mixture and concentrating to dryness, followed by solid-liquid extraction with an organic solvent in a Soxhlet extractor that

has a cartridge made out of a material compatible with said solvent, and eluting with a first eluent in a column selected ~~among~~ from the group consisting of filtration columns with cross-linked dextrane polymer fillers, filtration columns with acrylamide polymer fillers, filtration columns of active carbon ~~or~~ and active carbon-celite columns.

8. (currently amended) ~~Process~~ The method according to claim 7, ~~characterized in that~~ wherein the solvent is ethyl acetate.

9. (currently amended) ~~Process~~ The method according to claim 7, ~~characterized in that~~ wherein the solvent is used in an amount ~~comprised~~ between 10 ml and 25 ml per gram of initial xylose.

10. (currently amended) ~~Process~~ The method according to claim 7, ~~characterized in that~~ wherein the celite is used in an amount ~~comprised~~ between 1 g and 2 g per gram of initial xylose.

11. (currently amended) ~~Process~~ The method according to claim 7, ~~characterized in that~~ wherein the column is of active carbon-celite wherein the carbon is deactivated by adding 35% hydrochloric acid.

12. (currently amended) ~~Process~~ The method according to claim 11, ~~characterized in that~~ wherein the celite is used in an amount ~~comprised~~ between 0.5 g and 2 g of celite per gram of initial xylose.

13. (currently amended) ~~Process~~ The method according to claim 11, ~~characterized in that~~ wherein the active carbon is used in an amount ~~comprised~~ between 0.5 g and 2 g of active carbon per gram of initial xylose.

14. (currently amended) ~~Process~~ The method according to claim 7, ~~characterized in that~~ wherein said first eluent is used in an amount ~~comprised~~ between 5 ml and 25 ml per gram of initial xylose.

15. (currently amended) ~~Process~~ The method according to claim 11, ~~characterized in that~~ wherein the hydrochloric acid is used in an amount ~~comprised~~ between 0.5 ml and 1.5 ml per gram of initial xylose.

16. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein in ~~the fifth~~ step (v), the fourth reaction mixture is subjected to direct addition of at least a second eluent on the active carbon wherein the 4-O- β -D-galactopyranosyl-D-xylose is adsorbed on the active carbon and the second eluent is water followed by diluted isopropanol with a growing proportion in volume of isopropanol in successive steps.

17. (currently amended) ~~Process~~ The method according to claim 16, ~~characterized in that~~ wherein the proportion in volume of isopropanol is ~~comprised~~ between 1% and 3% in a first step, between 3% and 5% in a second step and between 5% and 7% in a third step.

18. (currently amended) ~~Process~~ The method according to claim 16, ~~characterized in that~~ wherein the active carbon is used in an amount ~~comprised~~ between 2 g and 4 g of active carbon per gram of initial xylose.

19. (currently amended) ~~Process~~ The method according to claim 16, ~~characterized in that~~ wherein the second eluent is used in a total amount ~~comprised~~ between 30 ml and 50 ml of second eluent per gram of initial xylose.

20. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the reaction is stopped by cooling the second reaction mixture at 0 ° C.

21. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the fourth reaction mixture is obtained by separating the aglyconic fragment from the β -D-galactopyranoside substrate by means of filtration.

22. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the proportion of D-xylose in the second reaction mixture is 7.5% by weight.
23. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the proportion of β -D-galactopyranoside in the second reaction mixture is 1.5% by weight.
24. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein 20 units of β -D-galactosidase per gram of β -D-galactopyranoside are added.
25. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the reaction medium also comprises at least a cosolvent medium selected ~~among~~ from the group consisting of dimethylsulfoxide, dimethylformamide, dioxane and mixtures thereof.
26. (currently amended) ~~Process~~ The method according to claim 25, ~~characterized in that~~ wherein the reaction medium comprises 20% by weight of the cosolvent medium.
27. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the reaction is carried out at a constant temperature.
28. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the reaction temperature is from -5 ° C. to 40 ° C.
29. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the reaction temperature is higher than the freezing temperature of the second mixture and lower than 0 ° C.
30. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that~~ wherein the reaction temperature is -5 ° C.

31. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the reaction temperature is room temperature.

32. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the reaction medium is buffered to a pH of 7.

33. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ in ~~the third~~ step (iii), the reaction is stopped by freezing the second reaction mixture at a temperature of -78 ° C.

34. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ in ~~the third~~ step (iii), the reaction is stopped by heating the second reaction mixture up to a temperature of 100 ° C.

35. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ in ~~the third~~ step (iii), the reaction is stopped by separating the β -D-galactosidase by ultrafiltration.

36. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the β -D-galactopiranoside substrate is selected ~~between~~ from the group consisting of o-nitrophenyl β -D-galactopiranoside and lactose.

37. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the β -D-galactosidase enzyme is E. coli β -D-galactosidase.

38. (currently amended) ~~Process~~ The method according to claim 1, ~~characterized in that wherein~~ the β -D-galactosidase enzyme is Kluyveramyces lactis β -D-galactosidase.

39. (currently amended) A 4-O- β -D-galactopyranosyl-D-xylose ~~characterized in that it has been~~ obtained by ~~means of the process method defined in~~ of claim 1.

40. (original) A composition for in vivo evaluation of intestinal lactase in humans, characterized in that it comprises a 4-O- β -D-galactopyranosyl-D-xylo- se obtained by means of the process defined in claim 1.

41. (original) A solution for the in vivo evaluation of intestinal lactase in humans, characterized in that it comprises a solution selected between aqueous solutions and saline solutions of a 4-O- β -D-galactopyranosyl-D-xylos- e obtained by means of the process defined in claim 1.

42. (original) Use of 4-O- β -D-galactopyranosyl-D-xylose prepared according to claim 1, in the preparation of a composition for in vivo evaluation of intestinal lactase in humans.

43. (original) Use of 4-O- β -D-galactopyranosyl-D-xylose prepared according to claim 1, in the preparation of a solution selected between saline solutions and aqueous solutions for in vivo evaluation of intestinal lactase in humans.

44. (original) Use according to claim 42, characterized in that the 4-O- β -D-galactopyranosyl-D-xylose is combined with pharmaceutically acceptable amounts of at least one additive selected from among stabilizers, protecting agents, flavoring agents, lactose, gelling agents, fluidizing agents and preservatives.